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454 Sequencing Is an Effective Method for Gap Closure in Microbial Whole Genome Shotgun Sequencing

Feng Chen, Jamie Jett, Edward Kirton, Eugene Goltsman, Vasanth Singan, Christopher Hack, Douglas Smith, and Paul Richardson

US DOE Joint Genome Institute
2800 Mitchell Dr., B400
Walnut Creek, CA 94598

At JGI, major efforts have been spent on sequencing through gaps in the assemblies generated by whole genome shotgun sequencing. Traditional shotgun sequencing is known to have difficulty in both cloning of A/T rich regions and sequencing of G/C rich regions. To help alleviate this problem we have applied the 454 sequencing platform as another tool for gap closure. Although 454 sequencing has been shown to have difficulty with homopolymer, it does not have the same biases as traditional sequencing. Therefore these two approaches together can be complementary. Different strategies have been tested for applying 454 sequencing in gap closure stage of WGS sequencing. Direct shotgun sequencing using 454 platform has been combined with traditional Sanger sequencing at the final assembly stage. We also developed a protocol in which gap-spanning clones are pooled, sequenced and assembled with 454 platform and the resulting contigs are added into their respective Sanger assemblies. The quality of the final assemblies from these different strategies was examined and compared. The regions only covered by 454 sequencing were studied and the sequencing features of these regions were analyzed. The base quality and assembly correctness of these regions were also assessed. Detailed results will be presented.

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